

On page 22, line 7 replace the paragraph beginning with “Figure 8” with the following:

**Figures 8A-8B:** BSP Promoter Sequence from -2184 to +237 (SEQ ID NO:1).

On page 30, lines 16-23, replace the paragraph beginning with “The present invention” with the following:

The present invention encompasses polynucleotide sequences comprising the 5' regulatory region, and transcriptionally active fragments thereof, of the BSP gene. In particular, the present invention provides polynucleotides comprising 907 bp, 1107 bp, 1418 bp, 1459 bp and 2253 bp sequences that are located within a BSP gene. Specifically, the polynucleotides comprise -838 bp through +69 bp, -1038 bp through +69 bp, -1349 bp through +69 bp, -1390 bp through +69 bp and -2184 bp through +69 bp, respectively, of the BSP sequence shown in Figures 8A-8B (SEQ ID NO:1). In various embodiments, the polynucleotide may be 5000, 4000, 3000, 2000, 1000 and preferably approximately 500 bp in length.

On page 31, lines 20-24, replace the paragraph beginning with “The nucleotide sequences” with the following:

The nucleotide sequences of the invention also include nucleotide sequences that have at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more nucleotide sequence identity to the nucleotide sequence depicted in Figures 8A-8B (SEQ ID NO:1), and/or transcriptionally active fragments thereof, which are capable of driving expression specifically within tumor and tissue cells with calcification potential.

On page 33, lines 1-11, replace the paragraph beginning with “Also encompassed within” with the following:

Also encompassed within the scope of the invention are various transcriptionally active fragments of this regulatory region. A “transcriptionally active” or “transcriptionally functional” fragment of the sequence depicted in Figures 8A-8B (SEQ ID NO:1) according to the present invention refers to a polynucleotide comprising a fragment of said polynucleotide which is functional as a regulatory region for expressing a recombinant

polypeptide or a recombinant polynucleotide in a recombinant cell host. For the purpose of the invention, a nucleic acid or polynucleotide is "transcriptionally active" as a regulatory region for expressing a recombinant polypeptide or a recombinant polynucleotide if said regulatory polynucleotide contains nucleotide sequences which contain transcriptional information, and such sequences are operably associated to nucleotide sequences which encode the desired polypeptide or the desired polynucleotide.

On page 33, line 31 to page 34, line 22, replace the paragraph beginning with "Also encompassed within" with the following:

Also encompassed within the scope of the invention are modifications of this nucleotide sequence without substantially affecting its transcriptional activities. Such modifications include additions, deletions and substitutions. In addition, any nucleotide sequence that selectively hybridizes to the complement of the sequence depicted in Figures 8A-8B (SEQ IDNO:1) under stringent conditions, and is capable of activating the expression of a coding sequence specifically within tumor and tissue cells with calcification potential is encompassed by the invention. Exemplary moderately stringent hybridization conditions are as follows: prehybridization of filters containing DNA is carried out for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 hours at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10<sup>6</sup> cpm of <sup>32</sup>P-labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography. Alternatively, exemplary conditions of high stringency are as follows: *e.g.*, hybridization to filter-bound DNA in 0.5 M NaHPO<sub>4</sub>, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. *et al.*, eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3). Other conditions of high stringency which may be used are well known in the art. In general, for probes between 14 and 70 nucleotides in length the melting temperature (T<sub>M</sub>) is calculated using the formula:

$T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log[\text{monovalent cations (molar)}]) + 0.41 (\% \text{ G+C}) - (500/N)$  where N is the length of the probe. If the hybridization is carried out in a solution containing formamide, the

melting temperature is calculated using the equation  $T_m(^{\circ}\text{C})=81.5+16.6(\log[\text{monovalent cations (molar)}])+0.41(\% \text{ G+C})-(0.61\% \text{ formamide})-(500/N)$  where N is the length of the probe. In general, hybridization is carried out at about 20-25 degrees below  $T_m$  (for DNA-DNA hybrids) or 10-15 degrees below  $T_m$  (for RNA-DNA hybrids).

On page 38, line 28 to page 39, line 13, replace the paragraph beginning with "Methods for assaying" with the following:

Methods for assaying promoter activity are well-known to those skilled in the art (see, *e.g.*, Sambrook *et al.*, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989). An example of a typical method that can be used involves a recombinant vector carrying a reporter gene and genomic sequences from the BSP sequence depicted in Figures 8A-8B (SEQ ID NO:1). Briefly, the expression of the reporter gene (for example, green fluorescent protein, luciferase,  $\beta$ -galactosidase or chloramphenicol acetyl transferase) is detected when placed under the control of a biologically active polynucleotide fragment. Genomic sequences located upstream of the first exon of the gene may be cloned into any suitable promoter reporter vector. For example, a number of commercially available vectors can be engineered to insert the BSP regulatory region of the invention for expression in mammalian host cells. Non-limiting examples of such vectors are pSEAP-Basic, pSEAP-Enhancer, p $\beta$ gal-Basic, p $\beta$ gal-Enhancer, or pEGFP-1 Promoter Reporter vectors (Clontech, Palo Alto, CA) or pGL2-basic or pGL3-basic promoterless luciferase reporter gene vector (Promega, Madison, WI). Each of these promoter reporter vectors include multiple cloning sites positioned upstream of a reporter gene encoding a readily assayable protein such as secreted alkaline phosphatase, green fluorescent protein, luciferase or  $\beta$ -galactosidase. The regulatory sequences of the BSP gene are inserted into the cloning sites upstream of the reporter gene in both orientations and introduced into an appropriate host cell. The level of reporter protein is assayed and compared to the level obtained with a vector lacking an insert in the cloning site. The presence of an elevated expression level in the vector containing the insert with respect the control vector indicates the presence of a promoter in the insert.

On page 66, lines 14-29, replace the paragraph beginning with "Construction of the" with the following: